

ABSTRACT OF THE DISCLOSURE

The invention relates to protein-protein interactions and methods for identifying interacting proteins and the amino acid sequence at the site of interaction. Using
5 overlapping hexapeptides that encode for the entire amino acid sequences of the linker domains of human P-glycoprotein gene 1 and 3 (HP-gp1 and HP-gp3), a direct and specific binding between P-gp1 and 3 linker domains and intracellular proteins was demonstrated. Three different stretches (⁶¹⁷EKGIYFKLVTM⁶²⁷,
⁶⁵⁸SRSSLIRKRSTRRSV^{RG}SQA⁶⁷⁷ and ⁶⁹⁴PVSFWRIMKLNLT⁷⁰⁶ for P-gp1 and
10 ⁶¹⁸LMKKEGVYFKLVNM⁶³¹, ⁶⁴⁸KAATRMAMPNGWKSRLFRHSTQKNLKNS⁶⁷⁴ and
⁶⁹⁵PVSFLKVLKLNKT⁶⁷⁷ for P-gp3) in linker domains bound to proteins with apparent molecular masses of ~80 kDa, 57 kDa and 30 kDa. The binding of the 57 kDa protein was further characterized. Purification and partial N-terminal amino acid sequencing of the 57 kDa protein showed that it encodes the N-terminal amino acids of alpha and beta-tubulins.
15 The method of the present invention was further validated with Annexin. The present invention thus demonstrates a novel concept whereby the interactions between two proteins are mediated by strings of few amino acids with high and repulsive binding energies, enabling the identification of high-affinity binding sites between any interacting proteins.